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***RESEARCH ARTICLE*.....**

# Efficacy of biofermenter on shrimp head waste using *Lactobacillus brevis* (MTCC 1750)

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**ABSTRACT.....** The shellfish processing industry in India generates about 8.5 million tonnes of waste per year which is rich in protein (40.37±0.74) with excellent amount of amino acid and can effectively be substituted in fish meal for feed preparation. The utilization of available protein in shrimp head meal by fishes is limited due to the presence of crude fibre (chitin). Fermentation can reduce this crude fibre by the breakdown of glycosidic bond between protein and chitin converting the product easily digestible. Fermentation of shrimp head waste in biofermenter reduces the fermentation time substantially as compared to conventional method.

**KEY WORDS.....** Shrimp head waste, Proximate composition, *Lactobacillus brevis*, Fermentation, Biofermenter

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## INTRODUCTION.....

Shrimp processing is one of the major food industries in India which are normally marketed as headless and often with exoskeleton removed. Only 40 per cent of the shrimp is edible and remaining 60 per cent considered as processing discards (Sindhu and Sherief, 2011). The solid waste generated in Indian shrimp processing industry was to the tune of 125,000 to 150,000 tonne per annum (Ramyadevi *et al*., 2012). These discards find very little practical application at present and are categorized as a major environmental contaminant. Effective utilization of this waste can resolve many of the environmental concerns facing the shellfish processors (Shahidi *et al*., 1992).

Continued production of the shrimp head waste without corresponding development of technology utilizing the waste has resulted in waste collection, disposal and pollution problems. The unused shell of shrimps is a good source of protein with excellent amino acid profile, fat and minerals and can offer a potential source for exploitation as fish feed (Nargis *et al*., 2006).

This abundant waste may pose a disposal problem and contributes to the overall cost of production. To solve this problem, it is important to transform waste into either silage or mill and use this material in the formulation of fish feed (Meyers, 1986). However, the use of shrimp head meal in the formulation of fish feed is not recommended due to its high fibre and ash contents, which results in the formation of weak pellets with poor stability in water. According to Fagbenro *et al.* (1997),

fibre and ash content could be reduced by transformation of shrimp head waste into silage by the process of fermentation. However, prompt preservation through lactic acid bacterial fermentation has been used successfully in fish feeds. Lactic acid bacterial fermentation is desirable as an alternative to cooking, sun drying and acid insolation as this process could reduce the chitin and ash content and consequently increase the protein, lipid and pigment concentration (Hall and De, 1994 and Fox *et al.*, 1994). The aim of the present study is to compare the fermentation of shrimp head waste using *Lactobacillus brevis* (MTCC 1750) following two methods *i.e.* conventional method and biofermenter.

## RESEARCH METHODS.....

Fresh shrimp waste (mainly head portion of *Penaeus monodon*) was collected from seafood export company Nezami Rekha Seafoods, Kolkata, West Bengal, India. The collected raw material were immediately transported to the laboratory in an insulated box in iced condition and stored at 4±1$^0$C using domestic refrigerator.

### Proximate composition :

The proximate composition of raw and fermented shrimp head waste were determined following the standard method (AOAC, 1995). For moisture content determination, the samples were heated overnight in an electric oven at 60$^0$C. For ash content, ground dried samples were heated for 5 hrs in an electric oven 525$^0$C. Total protein content was calculated my multiplying kjeldahl nitrogen by 6.25.

### Inoculum preparation :

Pure bacterial culture of *Lactobacillus brevis* (MTCC 1750) was maintained in Man Rosoga Shrape (MRS) Broth. The inoculum was prepared by adding a loop full of cells to MRS broth incubating at 37$^0$C for 48 hrs.

### Fermentation in conventional method :

Fermentation process was done following the method of Nwanna (2003). 500g of blended paste prepared from thoroughly washed raw material (shrimp head waste) was poured into a conical flask.75g @ 150g/kg of cane molasses and 50 ml water were added to the paste and sterilized in an autoclave maintained at 121$^0$C for 15 minutes. The material was inoculated with 25 ml @ 50ml/kg bacterial strain and allowed to ferment at 37$^0$C for 14 days.

### Fermentation in biofermenter:

4 kg shrimp head waste paste was poured into the fermentation chamber of the biofermenter. 600g @ 150g/kg of cane molasses and 400 ml water were added to the paste and sterilized at 121$^0$C for 15 minutes. 200 ml @ 50ml/kg bacterial strain was used to ferment for a period ranging from one to ten days at 37$^0$C temperature in anaerobic condition in order to assess the highest protein recovery.

Amino acid compositions of all the fermented products were analyzed according to the method described by Cohena *et al.* (1989).

### Standardization of fermentation period in biofermenter:

Sample was collected through the outlet of biofermenter every day in order to standardize the fermentation period depending upon the protein content.

### Drying of fermentation product :

Moisture content of the fermented product was estimated by drying in hot air oven at 60$^0$C taking the sample at an interval of two hours.

### Statistical analyses :

Data generated from the experiment were subjected to one way of analysis of variance using the SPSS (Statistical Package Computer, Software 1988 version Chicago Illinois, USA).

## RESEARCH FINDINGS AND ANALYSIS.....

The results obtained from the present investigation as well as relevant discussion have been summarized under the following heads :

### Proximate composition of raw shrimp head waste :

It is revealed from Table 1 that raw shrimp head

**Table 1: Proximate composition of shrimp head waste**

| Parameter | Protein (%) | Fat (%) | Ash (%) | Moisture (%) | Crude fibre (%) | NFE (%) |
|---|---|---|---|---|---|---|
| Value | 40.37±0.74 | 9.86±0.17 | 17.82±0.35 | 10.74±0.34 | 15.78±0.29 | 5.43 |

waste contains 40.37 per cent crude protein on dry weight basis which is in agreement with the result of Sachindra and Bhaskara (2008). Shrimp head waste contains 19.82 per cent ash duly supported by the result of Mizani and Aminlari (2007) and Tarafder *et al.* (2010). 15.78 per cent crude fibre was obtained from shrimp head waste in the present study.

**Proximate composition of fermented shrimp head waste :**

Considerable increase in nutrient content was noted in terms of the protein for fermented products. Protein and crude fibre content of 55.51 and 5.87 per cent were obtained in conventionally produced fermented product, respectively using *Lactobacillus brevis* (MTCC 1750) (Fig.1). Such similar result was obtained by Nwanna (2003) using *Lactobacillus planterum* taking heads of *Penaeusnotialis, P. duorarum, P. Kerathurus* and *Parapepaeus longirostris* as raw material.

The high protein and low fibre content are the indications of high digestibility. In the present study, protein content has increased by 37.48 per cent and decrease in crude fibre was to the extent of 62.80 per cent. Hydrolyzing chitin in shrimp waste by using crude chitinase extracellular from *Serrata marcescens* (shrimp waste hydrolysate) could decrease chitin content by 61.07 per cent and increased the protein content by 26.09 per cent which is in concurrence with the findings of the present study (Mahata *et al.*, 2008). It might be due to production of lactic acid which can break glycosidic bond between protein and chitin.

Fermented shrimp waste using *Lactobacillus brevis* (MTCC 1750) in biofermenter (at 37$^0$C for 6 days) contains 55.81 per cent protein (Fig.1). Cira *et al.* (2002) obtained protein content of 46.1 per cent in fermented shrimp waste using *Lactobacillus* sp. in column reactor at 30$^0$C after the fermentation period of 6 days. In the present study, raw shrimp head waste contains 17.82 per cent ash which has increased by 15.82 per cent and 14.08 per cent after fermentation in conventional method and using biofermenter, respectively. Demineralization of shrimp waste during the fermentation process could be the reason for the increase in ash content in the samples.

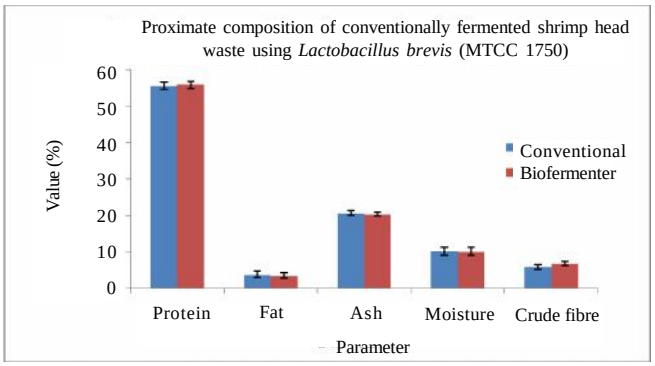


**Fig. 1 : Proximate composition of fermented shrimp head waste**

There was no significant difference in protein content in both the methods followed, but however, the time taken for fermentation in conventional method was 14 days as compared to 6 days in biofermenter. Therefore, the fermentation process following biofermenter may be considered better over conventional method.

**Table 2 : Essential amino acid composition (g/100g protein) of shrimp head waste fermented in conventional method and biofermenter**

| Name of amino acid | Amino acid composition | |
|---|---|---|
| | Conventional method | Biofermenter |
| Arginine | 2.73 | 2.32 |
| Histidine | 0.59 | 0.51 |
| Isoleucine | 1.79 | 1.56 |
| Leucine | 2.48 | 2.33 |
| Lysine | 2.03 | 2.28 |
| Methionine | 1.09 | 1.10 |
| Phenyialanine | 5.96 | 5.85 |
| Threonine | 2.54 | 2.36 |
| Tryyptophan | 0.49 | 0.41 |
| valine | 1.87 | 1.83 |

**Essential amino acid composition of fermented shrimp head waste :**

Fish proteins are of high nutritional quality and fairly balanced with respect to various essential amino acids. In order to fully evaluate the nutritive value of fish protein, it is desirable to establish its amino acid composition. This experiment was carried out to quantify the amino acid in fermented shrimp head waste following conventional method and biofermenter (Table 2). From the experimental result, it was observed that the content of phenylalanine was found to be more in fermented samples produced irrespective of the method followed with the availability of other essential amino acids in lesser quantity. The findings of the present study are in concurrence with the work of Nwanna (2003) working on fermented shrimp head waste meal. In amino acid composition, there was no significant difference in both the fermentation methods.

**Standardization of fermentation period :**

An attempt was made to standardise the process of fermentation using biofermenter because of inadequate information available pertaining to the process. Considering on the basis of protein content, standardisation of the fermentation process was done by drawing sample at one day interval. The result shows (Fig.2) that the protein content was highest on 6$^{th}$ day which is at par with the finding of Cira *et al*. (2002) working on fermented shrimp waste using *Lactobacillus* sp. in column reactor. In the present experiment protein percentage was highest on 6$^{th}$ day with marginal reduction in the subsequent days of fermentation. Hence, period of fermentation for 6 days may be considered as ideal using a biofermenter.

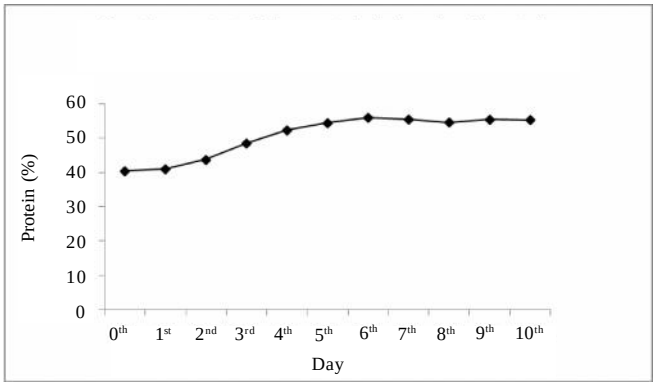


**Fig. 2 : Protein content of fermented shrimp head waste in biofermenter using *Lactobacillus brevis* (MTCC 1750)**

**Drying of fermented products :**

Fermented products need to be dried for its use as fish feed. In the present experiment, moisture content of 73.09 per cent was found in fermented shrimp head waste necessitating to reduce it to an acceptable level. In order to standardise the process, the material was subjected to drying at 60$^0$C and sample was drawn at 2 hrs interval (Fig.3). It was observed that drying period of 14 hrs at given temperature was most ideal for the product duly supported by the findings of Amar *et al.* (2006).

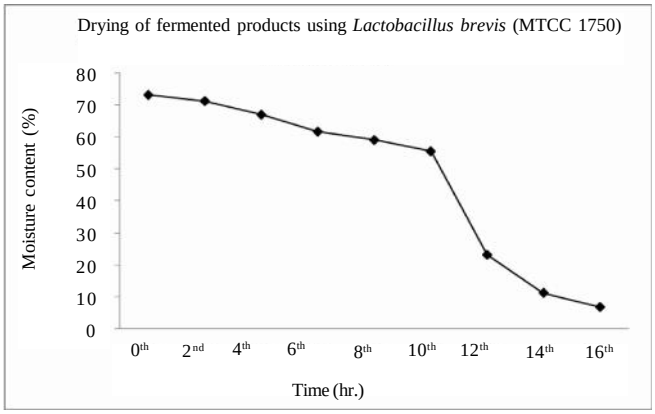


**Fig. 3 : Drying of fermented products (in 2 hrs. interval, in % of moisture)**

**Conclusion :**

The development of aquaculture is hampered by inadequate supply of feedstuffs particularly fish meal which is scarce and expensive stimulating the evaluation of a variety of alternative dietary protein sources. Continued production of shrimp head waste containing high amount of protein with excellent amino acid profile without corresponding development of the technology has attracted the application of different processing methods. Fermentation is a better process of shrimp head waste utilization than others for the preparation of fish feed. From the result of the present study, it can be concluded that the fermentation using a biofermenter is better over conventional method primarily considering the process time.

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